Chlamydomonas axonemal tubulin despite greater than 85% similarity in primary structure. Finally, we found that the instantaneous translocation speed of microtubules in the gliding assay is unsteady. Our analysis suggests that the source of this unsteadiness may arise from the same mechanochemical properties of dynein that have been predicted to be sufficient for coordination. Together these results suggest that the interactions between dynein and tubulin are important factors in axonemal dynein coordination.

1888-Pos Board B658
Characterization of the Beat of Chlamydomonas Axonemes
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The axoneme is an evolutionarily conserved mechanical apparatus within cilia and flagella made up of microtubules, several different axonemal dynein heavy chains, and accessory proteins. The aim of this study was to determine how the shape of the axonemal beat depends on the dynein composition and the chemical and mechanical properties of the axoneme.
We used high-speed microscopy to record the shapes of beating Chlamydomonas axonemes. Through image analysis we measured the amplitudes of the Fourier modes that characterize the shapes of regularly beating axonemes. We then used the Fourier description to compare the waveforms of wildtype and mutant axonemes. By changing chemical conditions (e.g. ATP concentration) we determined how the shape correlates with the beat frequency and by comparing the waveforms of intact cells and isolated, reactivated axonemes, we determined how the shape correlates with hydrodynamic loading and boundary conditions.
We anticipate that these data will provide insight into how the axonemal beat depends on the chemomechanical interactions between the dynein molecules.

Actin & Actin-binding Proteins

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Expression of Recombinant Beta-Actin in the High-Density Insect Cell Culture
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Actin is the most abundant protein in the cytoplasm of most eukaryotic cells, which plays important roles in such processes as muscle contraction, cell division, morphogenesis, organelle positioning, and vesicle trafficking. In our previous study, we reported the expression method of recombinant beta-actin at high cell density in a shaking Erlenmeyer flask. In the insect cell culture at early stationary phase (1-1.2 x 10^7 cells per ml), additional nutrients or medium exchange at time of infection restored the expression level of beta-actin per cell equivalent to that in the cell culture at mid-logarithmic phase (1-2 x 10^6 cells per ml). Therefore, the volumetric yield of recombinant beta-actin, which was determined by the maximal cell density and the yield of recombinant protein per cell, was at least 6.5-fold higher than compared to the manufacturer's protocol. The present study further developed the expression method of recombinant beta-actin at the density of 1.75 x 10^7 cells per ml, which is ~1.5-fold higher than the cell density at stationary phase. This method requires medium exchange plus additional nutrients and air ventilation after infection. The volumetric yield of recombinant beta-actin reached 200 mg per liter of insect cell culture. Here we report in detail the effective method for the production of recombinant beta-actin and the biological and the biochemical properties of recombinant beta-actin.

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Actin Polymerization Correlates with the Flattening of Actin Molecule
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Actin plays the fundamental roles in a variety of cell functions in eukaryotic cells. Actin is a polymerizable protein and in two forms; monomeric G-actin and fibrous F-actin. The polymerization - depolymerization cycle drives essential cell processes, such as cell locomotion and cell division. For understanding of these processes, atomic structures for G-actin and F-actin are essential. Recently, we proposed the atomic model for F-actin structure, and found that actin is in the twisted form in a monomer, and in the untwisted form in a filament. The question when the transition from the twisted to the untwisted form occurs in filament formation remains elusive.
In this study, to access the question, we prepared two actin mutants by the use of the insect cell expression system we developed. We focused on the loop A108-P112 crucial for the flattening transition. We introduced site-directed mutations at 108th Ala to Gly; A108G, and 109th Pro to Ala; P109A in the loop. Both mutants were purified to a high homogeneity as well as the wild-type, and were measured the polymerization activity and the actin ATPase activity relevant to polymerization.
We demonstrated that A108G had both reduced elongation and ATP hydrolysis rates, and P109A had both accelerated ones on polymerization. These results indicate that A108G and P109A in the loop A108-P112 modulated the polymerization activity of the actin mutants, but did not affect the intrinsic ATPase activity relevant to polymerization. The mutant analyses of A108G and P109A suggest that the polymerization event correlates directly with the flattening of actin molecule.

1891-Pos Board B661
Models of Heterogenous Actin Filaments
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Actin filaments consist of actin proteins, which are abundant in eukaryotic cells. These filaments form mesh-like structures to provide mechanical support and determine the shape of cells. Actin filaments also play important roles in cell mobility, cell division, endocytosis and intracellular transportation. In all these cellular processes, the mechanical properties of the actin filaments are crucial to facilitate cell dynamics. The mechanical properties are determined by the actin's inter- and intra- subunit interactions. To obtain these interactions, we constructed heterogenous coarse-grained actin filaments. The coarse-grained (CG) beads were obtained using essential dynamics coarse-graining (EDCG) method to analyze molecular simulation data of actin filaments. Two kinds of EDCG models were built, a uniform-CG filament and a hetero-CG filament, corresponding to using the same or different coarse-grained representations for each actin subunit, respectively. We found that the hetero-CG filament has a lower free energy than the uniform-CG filament, which suggests the hetero-CG representation is energetically more favorable at the MD simulation time scale. We further quantified the persistence length and torsional stiffness of CG filaments. This work enhances the understanding of the interactions between and within actin subunits and explores how these interactions affect the mechanical properties of actin filaments.

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Specific Ion Efects on Actin Filament Stability and Flexural Rigidity
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It is well established that ions influence actin filament assembly and flexural rigidity. However, a molecular relationship between actin filament thermodynamic stability and bending mechanics is lacking. Here, we evaluate the linkage of cation interactions with actin polymerization and the flexural rigidity of actin filaments. Our results reveal that the thermodynamic stability and flexural rigidity of actin filaments increase with cation concentrations in a manner that suggests specific binding of ions to filaments as opposed to electrostatic screening effects. Using structural bioinformatics, we identify two distinct potential filament specific cation-binding sites that explain how specific cation binding to them could influence polymerization and the flexural rigidity of filaments. Consistent with this prediction, site-specific substitution of a charged amino acid residue at one of the sites modulates the [cation]-dependence of filament flexural rigidity. Substitution at the second site dramatically increases the polymerization critical concentration.

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Molecular Dynamics Analysis of Coupling Behaviors Between Extension and Torsion of Actin Filaments
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Actin filaments are semi-flexible polymers that display mechanical stretching, twisting and bending motions, and have a double helical structure consisting of globular actin molecules, that induces coupling motions between stretching and